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Effects of Sample Storage on a Copper-Spiked Freshwater Sediment

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16. ABSTRACT

Freshwater sediment from an [illegible] lake was spiked with copper in the laboratory to study the effect of storage on [illegible] toxicity. Peat moss was added to enhance the carbon content of a portion of the sediment. One-half of the sediment samples were stored at 5°C while the other half were frozen (-20°C). At intervals up to 25 weeks, samples were brought to 20°C and 48-h toxicity tests were conducted in 1-liter beakers (200 ml sediment, 800 ml water) using the cladoceran Daphnia magna Straus.

Copper released into the water from the sediment stored at 5°C increased over the first 8 weeks of storage, then decreased. Freezing the sediment attenuated the release of total soluble copper into the overlying water, resulting in low or no toxicity to daphnids. Based on total copper concentrations in the overlying test water, LC50s ranged from 7 to 44 µg/L in sediments without peat and from 52 to 96 µg/L in sediments with added peat. Soluble copper LC50s for the same test waters ranged from 3 to 18 and from 25 to 68 µg/L, respectively. No LC50s could be calculated from the frozen sediment tests because of low mortality. Freezing is not recommended as a storage procedure because of its undependability in preserving sediment integrity for toxicity tests.

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EFFECTS OF SAMPLE STORAGE ON A COPPER-SPIKED FRESHWATER SEDIMENT

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Abstract—Freshwater sediment from an Oregon lake was spiked with copper in the laboratory to study the effect of storage on copper toxicity. Peat moss was added to enhance the carbon content of a portion of the sediment. One-half of the sediment samples were stored at 5°C while the other half were frozen (−20°C). At intervals up to 25 weeks, samples were brought to 20°C and 48-h toxicity tests were conducted in 1-liter beakers (200 ml sediment, 800 ml water) using the cladoceran *Daphnia magna* Straus.

Copper released into the water from the sediment stored at 5°C increased over the first 8 weeks of storage, then decreased. Freezing the sediment attenuated the release of total and soluble copper into the overlying water, resulting in low or no toxicity to daphnids. Based on total copper concentrations in the overlying test water, LC50s ranged from 7 to 44 μg/L in sediments without peat and from 52 to 96 μg/L in sediments with added peat. Soluble copper LC50s for the same test waters ranged from 3 to 18 and from 25 to 68 μg/L, respectively. No LC50s could be calculated from the frozen sediment tests because of low mortality. Freezing is not recommended as a storage procedure because of its undependability in preserving sediment integrity for toxicity tests.

Keywords—Freshwater sediment Toxicity tests Sample storage *Daphnia* Copper

INTRODUCTION

Sediments are a dynamic element of aquatic ecosystems, yet with respect to toxic substances, they are less well studied than the overlying waters. Pollutants can accumulate in the sediment and subsequently be released back into the water column. Thus, availability of sediment-bound pollutants is of importance because of their potentially adverse effects. Interest in sediment research has increased, especially in the areas of sediment/water exchange, ecological effects of dredging and disposal, hazardous waste assessment and sediment contamination criteria. These activities, and research in related areas, emphasize the need to standardize sediment handling methods.

As part of the sediment toxicity research program at the Environmental Protection Agency (EPA) Corvallis Environmental Research Laboratory (CERL), we are investigating sediment handling techniques. We compared cold versus frozen storage of a copper-spiked sediment, containing two levels of organic matter, for a period of 25 weeks, investigating the relationship of storage time and organic matter to copper availability and acute lethality to *Daphnia magna*.

METHODS

More than 110 liters of sediment was collected from Porter Lake, 13 km south of Corvallis, Oregon, at a water depth of about 3 m. An Ekman grab was used. Water overlying the sediment in the sampler was poured off during collection. All the sediment was thoroughly mixed and refrigerated at 5°C for no longer than 48 h before it was spiked with copper.

Sediment subsamples were spiked and mixed in 4-liter, wide-mouthed polyethylene containers; two were needed for each desired nominal copper concentration. Only 2.5 liters of sediment destined for frozen storage was placed in each container, to allow room for adequate mixing. Slightly more, 2.75 liters, was placed in containers for cold storage. This increased amount allowed sufficient sediment for an initial prestorage bioassay.

Peat moss (Radel, Inc., Likely, CA) ground and screened through a 0.83-mm opening sieve, was added at a concentration of 220 g/kg dry solids to enhance the organic content of one-half

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of the stored sediment. Sediment with no added peat moss contained 5.8% organic matter; the addition of peat increased this value to 14.4%.

Adsorption isotherms previously developed in our laboratory (unpublished data) were used to calculate the amount of copper needed in the sediment to obtain the desired concentration in the water. Nominal sediment levels for copper were 375, 750, 1,100 and 1,500 mg/kg in sediment with no peat, and 750, 1,400, 1,700 and 2,000 mg/kg in sediment with added peat. Sediment with no added peat destined for frozen storage was spiked, inadvertently, at lower levels: 300, 600, 900 and 1,200 mg/kg. The copper was contained in a solution of reagent-grade cupric chloride ($CuCl_2 \cdot 2H_2O$) at a strength of 20 mg Cu/ml. The background concentration of copper in Porter Lake sediment was 49 ± 7 mg/kg (mean ± SD).

The peat was added first, stirred in, and then followed by the appropriate copper solution, ranging from 19 to 77 ml per mixing container for sediment with no peat and from 50 to 126 ml for peat-added sediments. After the copper was added, the air space above the sediment was purged with nitrogen for 30 s before the container was closed. The containers were then shaken for 2 h at approximately 5 oscillations per second.

Aliquots (500 ml) were poured into flexible polyethylene, 1-liter Cubitainers (Hedwin Corporation, Spartanburg, SC), which were squeezed to expel all air before capping. The Cubitainers were wrapped with a single thickness of aluminum foil for cold storage at 5°C and with a double thickness for frozen storage at −20°C. All mixing and storage containers were acid-rinsed with HNO_3 before use. The sediments destined for frozen storage were prepared 2 d after the cold-stored sediments were.

When sediments were needed for testing (1, 3, 8, 12, 17 and 25 weeks), one Cubitainer of each copper concentration and sediment type was removed from storage. Cold sediments were allowed to warm to room temperature (approx. 20°C); frozen sediments were brought to room temperature in a few hours in a flowing-water bath. Each Cubitainer contained sufficient sediment for a duplicate test.

Control sediments were prepared in the same manner as were the test sediments, but without the addition of copper. A freshly collected control sediment was also tested at each time interval.

Dilution water

Test water was obtained from a laboratory well located near the Willamette River at the Western Fish Toxicology Station, a CERL facility. This water is characteristically soft, with a calculated total hardness of 22 mg/L as $CaCO_3$, a calculated alkalinity of 26 mg/L as $CaCO_3$ and a specific conductance of 67 μS/cm. Trace metal concentrations are at or below detection limits [1].

Test organisms

Daphnia magna Straus were obtained from a stock culture maintained at the Toxicology Station. The culture was originally obtained in 1971 from the U.S. EPA Environmental Research Laboratory in Duluth, Minnesota. Five-d-old organisms were used, and were fed the alga *Selenastrum capricornutum* Printz daily before testing. Details of the rearing methods can be found in Nebeker et al. [2].

Test conditions

The toxicity tests were conducted in duplicate in 1-liter beakers. Two hundred milliliters of sediment was placed in each beaker and 800 ml of dilution water was then added; a baffle was used to minimize disturbance. The beakers were left undisturbed for the next 5 d, allowing particulates to settle and sediment and water to interact. Two hours before the daphnids were added, gentle aeration was started using glass-tipped air lines. Ten or 15 test organisms were placed in each beaker, and survivors were counted 48 h later. Organisms were not fed during the test. Dissolved oxygen and pH were monitored throughout the setup and testing phases.

Tests were conducted in a controlled environment at 20 ± 0.5°C. A photoperiod of 16 h light: 8 h dark was maintained with cool-white fluorescent lights, giving an illuminance of about 430 lux at benchtop.

Analytical procedures

Sediment grain size was determined by U.S. EPA methods [3]. Particle size, as percent sand, silt, and clay, was determined by the hydrometer method of Day [4]. Percent organic matter was measured by the Walkley and Black titration method, as reported by Berg and Gardner [5]. Total organic carbon (TOC) in the water samples was determined by U.S. EPA methods [6]. U.S. EPA reference samples were analyzed; recoveries were 100 and 120% at levels of 4.1 and 6.1 mg/L, respectively. The precision of the TOC measurements in water was ±0.2 mg/L. Chemical analyses were conducted on pooled samples from each set of replicates. Following acid extraction [7],

metal concentrations in the sediment were determined by atomic absorption spectrophotometry using flame or graphite furnace techniques [8] and inductively coupled plasma-atomic emission spectrometric (ICPAES) methods [9]. Metals in the water column were analyzed for total and soluble levels by ICPAES methods. Soluble copper was defined as the portion remaining in solution after centrifugation at 20,000 rpm (48,200 *g*) for 10 min. Copper measurements by ICPAES were compared with measurements by atomic absorption and were not significantly different.

The precision of copper measurement in water was ±10 μg/L or ±10% of higher values, i.e., greater than 100 μg/L. Recovery of spiked samples exceeded 90%. The U.S. EPA digested municipal sludge sample No. 3492 was analyzed concurrently with the sediment samples to verify efficiency of digestion procedures and to determine recovery on a standardized sample. Recovery was 99% of the standard's reported value.

Statistical analysis

Mortality data from the two replicates were pooled prior to the calculation of LC50s. The trimmed Spearman-Karber method was used to calculate LC50s and their 95% confidence intervals [10]. Differences between mean particle sizes were tested with a *t* distribution. Relationships between chemical and biological parameters were analyzed with correlation and linear regression techniques.

RESULTS

There were observable differences between the cold-stored and the frozen-stored sediments after they were warmed. The frozen-stored material was less viscous than the semigelatinous cold-stored sediments. The frozen-stored sediments settled and decreased in volume from the original 200 ml to 100 to 150 ml soon after the test water was added to the beakers. The water above the sediments that had been frozen rapidly became turbid and had a yellowish cast. The volume of the cold-stored sediments remained at 200 ml and the associated water was clear.

At the beginning of most of the tests, turbidity was measured in four to five beakers containing either cold-stored or frozen-stored sediments. Turbidity ranged from 4 to 9 JTU in the water in contact with cold-stored sediment and from 33 to 45 JTU in the water above the frozen-stored sediments. The sand, silt and clay components of Porter Lake sediment were approximately 20, 34 and 35%, respectively. The addition of peat resulted in an approximate composition of 21, 40 and 39%, respectively.

Particle size distribution was measured in samples collected from Porter Lake just before and just after these tests. More than 70% of the material passed through a 63-μm mesh sieve in these two samples (Table 1). Particle size was measured again at the conclusion of the preservation tests. Freezing resulted in a significant increase in the material retained on the 850-μm and 250-μm mesh

Table 1. Porter Lake sediment size analysis

Sediment	% retained on mesh				
	10 (1,980 μm)	20 (850 μm)	60 (250 μm)	230 (63 μm)	% passed (230 mesh)
Fresh					
07/29/83	0	0	1.0 ± 0.2	22.2 ± 1.1	76.9 ± 1.1
04/05/84	1.3 ± 0.9	0.7 ± 0.5	3.1 ± 0.9	24.1 ± 0.6	70.0 ± 0.7
	NP	NP	S	NS	S
Storage for 29 weeks (collected 09/15/83)					
No peat					
Cold	1.7 ± 0.5	1.3 ± 0.3	3.0 ± 0.6	24.9 ± 0.5	69.0 ± 0.8
Frozen	1.8 ± 0.8	3.5 ± 1.0	16.5 ± 2.2	23.6 ± 4.9	54.6 ± 1.8
	NS	S	S	NS	S
Peat					
Cold	1.1 ± 0.8	0.9 ± 0.6	15.1 ± 0.2	28.5 ± 1.5	54.4 ± 0.7
Frozen	1.4 ± 0.7	4.6 ± 0.5	20.7 ± 0.9	29.6 ± 0.8	43.7 ± 1.4
	NS	S	S	NS	S

Values are means ±SD; $n = 3$. Significance between means ($\alpha = 0.05$): NP, *t* test not possible; NS, not significant; S, significant.

Table 2. Results of 48-h acute copper toxicity bioassays

Week	Nominal bulk (mg/kg)	Total[a] (μg/L)	Soluble[a] (μg/L)	pH[a]	TOC[a]	*Daphnia* Dead/Total	*Daphnia* % mortality	48-h LC50 (μg/L; 95% C.I.) Total	48-h LC50 (μg/L; 95% C.I.) Soluble
No peat									
0	375	12	0	7.4	2.7	0/30	0		
	750	11	0	7.2	1.5	0/30	0	20	8[b]
	1,100	12	9	6.7	0.7	19/30	63	(15–26)	(5–13)
	1,500	210	210	5.3	0.8	30/30	100		
1	375	0	0	7.4	3.2	0/20	0		
	750	0	0	7.2	2.0	0/20	0	7[b]	7[b]
	1,100	0	0	6.8	1.7	5/20	25	(4–12)	(4–11)
	1,500	180	170	5.6	1.4	20/20	100		
3	375	10	9	7.2	1.1	1/30	3		
	750	14	9	6.9	0.3	1/30	3	22	16
	1,100	37	30	6.4	0.3	30/30	100	(22–23)	(15–17)
	1,500	410	390	4.6	<0.2[c]	30/30	100		
8	375	28	0	7.0	—	0/30	0		
	750	31	0	6.8	—	16/30	53	44	3[b]
	1,100	160	150	5.2	—	30/30	100	(38–52)	(2–5)
	1,500	590	590	4.2	—	30/30	100		
12	375	20	20	6.8	1.6	0/30	0		
	750	17	15	6.7	1.0	25/30	83	20	18
	1,100	47	36	6.5	0.6	30/30	100	(19–21)	(17–19)
	1,500	300	300	5.6	0.4	30/30	100		
17	375	13	6	6.9	2.1	0/30	0		
	750	14	8	6.8	1.4	14/30	47	18	10
	1,100	21	12	6.8	1.0	25/30	83	(16–21)	(9–12)
	1,500	105	76	6.6	1.0	30/30	100		
25	375	14	10	7.1	<2.0[c]	2/30	7		
	750	10	10	7.0	—	2/30	7	>17	>15
	1,100	10	10	6.7	<2.0	0/30	0		
	1,500	17	15	6.6	<2.0	0/15	0		

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sieves and a significant decrease in the material that passed through the 63-μm sieve in sediments both with and without peat ($\alpha = 0.05$).

Correlations between nominal bulk copper and measured bulk copper were high; r^2 values for the four combinations of sediment and storage ranged from 69 to 90%.

Total and soluble concentrations of copper in the bioassay water were virtually the same in all tests (Table 2). Copper in the water from the cold-stored sediments with the two highest bulk copper concentrations increased sharply for 8 weeks, then decreased until the end of the experiment. As an example, in the sediment without peat spiked to obtain a nominal copper concentration of 1,500 mg/kg, total copper concentration started at 210 μg/L, decreased slightly at 1 week, increased to 590 μg/L at 8 weeks and then dropped to 17 μg/L at 25 weeks. Copper in the water from the two lowest bulk sediment concentrations followed the same pattern but with less variation. The addition of peat attenuated the release of total and soluble copper into the water column. Copper concentrations in the water were lower in tests in which the sediment contained peat than in those without peat.

Freezing dramatically decreased or virtually eliminated total and soluble copper in the water column (Table 3). Soluble copper concentrations in the waters changed little over time. They were generally low, ranging from 0 to 20 μg/L in water over sediments without peat and from 0 to 49 μg/L in water over sediments with peat added. Total copper values in bioassay water were more variable, especially in tests over sediments with added peat, in which a high value of about 250

in cold-stored sediments with and without added peat

Week	Nominal bulk (mg/kg)	Total[a] (μg/L)	Soluble[a] (μg/L)	pH[a]	TOC[a]	*Daphnia* Dead/Total	*Daphnia* % mortality	48-h LC50 (μg/L; 95% C.L.) Total	48-h LC50 (μg/L; 95% C.L.) Soluble
Peat									
0	750	0	0	7.0	12	0/30	0		
	1,400	46	26	6.8	10	0/30	0	52[b]	37[b]
	1,700	34	27	6.5	8.7	22/30	73	(44–61)	(30–46)
	2,000	360	330	5.2	9.2	30/30	100		
1	750	48	6	7.2	11	0/20	0		
	1,400	25	20	6.8	6.7	1/20	5	51	38
	1,700	76	54	6.6	5.4	16/20	80	(39–66)	(29–50)
	2,000	210	180	6.2	4.3	18/20	90		
3	750	41	22	7.0	9.4	0/30	0		
	1,400	77	29	6.8	6.3	0/30	0	96	68
	1,700	71	50	6.6	5.0	7/30	23	(91–101)	(60–76)
	2,000	150	130	6.3	5.0	30/30	100		
8	750	43	30	6.8	—	4/30	13		
	1,400	59	41	6.4	—	30/30	100	49	34
	1,700	160	130	6.0	—	30/30	100	(48–50)	(33–35)
	2,000	460	385	4.8	—	30/30	100		
12	750	22	20	6.9	3.0	0/30	0		
	1,400	29	18	6.9	3.5	1/30	3	59	46
	1,700	49	41	6.7	—	13/30	43	(51–69)	(39–54)
	2,000	150	120	6.6	3.0	30/30	100		
17	750	24	16	7.1	5.0	1/30	3		
	1,400	24	12	7.0	5.0	0/30	0	59	25
	1,700	37	17	7.0	4.0	2/30	7	(50–71)	(21–28)
	2,000	64	26	6.9	4.0	17/30	57		
25	750	20	20	7.0	<2.0[c]	1/30	3		
	1,400	18	18	6.8	<2.0	1/30	3	>34	>30
	1,700	20	20	6.8	<2.0	1/30	3		
	2,000	34	30	6.8	<2.0	1/30	3		

TOC, total organic carbon.
[a]Final values.
[b]Estimate based on zero dosages used in Spearman-Karber calculations.
[c]Variability in detection level, as indicated by <, is a function of sample size.

μg/L occurred in the water above the 1,700 mg/kg nominally spiked sediment at 8 weeks.

TOC in the water column (Tables 2 and 3) was higher in those vessels that contained frozen sediment. Maximum water concentrations were 16 mg/L in vessels containing peat-added sediments. TOC in the water column over the cold-stored sediments with peat decreased during the 25-week storage period.

The pH of most of the bioassay waters increased slightly during the testing phase. Measurements of pH were begun 3 to 4 d after the water and sediment had been added to the beakers and were continued daily until the end of the tests. Exceptions to this general increase in pH occurred in the bioassays using cold-stored sediments spiked with 1,500 and 2,000 mg/kg Cu (Table 2). In approximately three-quarters of the tests using sediments with no peat at 1,500 mg/kg Cu and approximately one-quarter of the tests using sediments with peat at 2,000 mg/kg Cu, the pH dropped below 6.0. This depression in pH generally did not occur until the last days of a particular bioassay experiment. The lowest final pH values were recorded for sediments stored for 3 and 8 weeks. Final pH values for the water over cold-stored sediment ranged from 4.2 to 7.4, while final pH values for the water over frozen-stored sediments were higher, ranging from 6.8 to 7.7. The large pH variations observed in the water over cold-stored samples were not evident in the water over frozen-stored samples (Table 3).

Daphnia mortality between replicate beakers was similar. Control mortality was low, averaging only 1.6 ± 3.9% (mean ± SD) for all tests. *Daphnia* mortality (Table 2) ranged from 0 to 100% in

Table 3. Results of 48-h acute copper toxicity bioassays

Week	Nominal bulk (mg/kg)	Total* (μg/L)	Soluble* (μg/L)	pH*	TOC*	*Daphnia* Dead/Total	*Daphnia* % mortality	48-h LC50 (μg/L; 95% C.I.) Total	48-h LC50 (μg/L; 95% C.I.) Soluble
No peat									
1	300	0	0	7.7	6.0	0/20	0		
	600	32	0	7.4	7.3	0/20	0	>32	>0
	900	0	0	7.4	3.9	0/20	0		
	1,200	0	0	7.2	3.1	0/20	0		
3	300	10	10	7.6	6.2	0/30	0		
	600	7	0	7.4	5.0	0/30	0	>21	>10
	900	12	0	7.2	4.8	1/30	3		
	1,200	21	0	7.0	4.5	2/30	7		
8	300	14	0	7.5	—	0/30	0		
	600	0	0	7.4	—	0/30	0	>60	>0
	900	0	0	7.3	—	1/30	3		
	1,200	60	0	7.2	—	2/30	7		
12	300	9	0	7.6	8.7	0/30	0		
	600	11	10	7.5	5.4	0/30	0	>21	>16
	900	21	16	7.5	4.4	1/30	3		
	1,200	10	10	7.3	4.3	0/30	0		
17	300	0	0	7.6	2.5	0/30	0		
	600	7	0	7.6	2.8	0/30	0	>7	>6
	900	6	6	7.5	5.1	0/30	0		
	1,200	7	6	7.3	4.0	1/30	3		
25	300	0	0	7.6	<2.0	0/30	0		
	600	0	0	7.7	<2.0	0/30	0	>10	>10
	900	10	10	7.6	<2.0	0/30	0		
	1,200	0	0	7.4	<2.0	0/30	0		

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cold-stored sediment bioassay tests with both levels of organic matter. The mortality was much lower in the frozen-stored sediment tests, a maximum of 23%, with most other values below 10% (Table 3). In cold-stored sediment tests, there was a trend of increasing mortality through 12 weeks, followed by a decline (Table 2).

Water LC50s (Table 2) based on total copper in the cold-stored sediment tests ranged from 52 to 96 μg/L for those with peat and from 7 to 44 μg/L for those without peat. Similarly, LC50s based on soluble copper ranged from 25 to 68 μg/L for those with peat and from 3 to 18 μg/L for those without peat. There were no significant trends in LC50s over time. No values for frozen-stored sediment could be calculated because of insufficient mortality.

DISCUSSION

Little information is available regarding the effects of freezing on the toxicity of contaminated freshwater sediments. Plumb [11] recommended storage at 4°C and storage times of less than 1 week for bioavailability testing of sediment samples in general.

Lee and Jones [12], in a discussion of an article by Laskowski-Hoke and Prater [13], stated: "Because the authors froze sediment samples, the data itself may not be valid and, consequently, all interpretations based on that data are suspect." Laskowski-Hoke and Prater [14] responded that "freezing may alter contaminant availability, although neither of us has seen in [*sic*] a published article dealing with this aspect of sediment chemistry."

Dillon [15] tested the toxicity of estuarine sediments in seawater bioassays with the mysid shrimp *Mysidopsis bahia*. The sediments were stored at −22°C, 4°C and 25°C for periods of up to 20 weeks. Sediments stored at 4°C and −22°C exhibited similar toxicities after storage for 4 weeks or more. Sediments stored at 4°C generally increased in toxicity until 12 weeks. A subsequent decrease in toxicity coincided with an increased resistance of newly hatched shrimp to a standard reference toxicant.

in frozen-stored sediments with and without added peat

Week	Nominal bulk (mg/kg)	Total[a] (μg/L)	Soluble[a] (μg/L)	pH[a]	TOC[a]	*Daphnia* Dead/Total	*Daphnia* % mortality	48-h LC50 (μg/L; 95% C.I.) Total	48-h LC50 (μg/L; 95% C.I.) Soluble
Peat									
1	750	20	20	7.5	15	0/20	0		
	1,400	20	20	7.1	13	0/20	0	>66	>30
	1,700	66	30	6.8	12	0/20	0		
	2,000	20	20	6.8	10	0/20	0		
3	750	24	16	7.4	16	1/30	3		
	1,400	17	8	7.0	14	0/30	0	>120	>40
	1,700	46	40	6.9	12	0/30	0		
	2,000	120	28	6.8	14	7/30	23		
8	750	45	14	7.4	—	0/30	0		
	1,400	14	9	7.2	—	1/30	3	>250	>14
	1,700	250	10	7.0	—	1/30	3		
	2,000	19	0	6.8	—	2/30	7		
12	750	40	40	7.4	10	0/30	0		
	1,400	30	30	7.3	10	0/30	0	>49	>49
	1,700	49	49	7.2	12	0/30	0		
	2,000	39	36	6.8	12	0/30	0		
17	750	30	27	7.6	16	0/30	0		
	1,400	32	26	7.4	15	0/30	0	>33	>27
	1,700	33	27	7.4	14	0/30	0		
	2,000	30	30	7.2	13	0/30	0		
25	750	25	24	7.6	<2.0	5/30	17		
	1,400	22	7	7.3	3.8	0/30	0	>35	>24
	1,700	35	8	7.2	14	0/15	0		
	2,000	27	13	7.2	14	2/30	7		

TOC, total organic carbon.
[a]Final values.

Variable pH levels may have influenced our test results. While low final pH values and high mortalities occurred together in many cases, high mortality (50 to 100%) was also recorded at pH 6.7 to 6.8 in the cold-stored sediment tests without peat and from 6.6 to 6.9 in the cold-stored sediment tests with peat.

Low pH and oxic water conditions may favor the presence of cupric ion, as pointed out by Stella and Ganzerli-Valentini [16]. They indicated that, at higher total copper concentrations, precipitation of copper occurred at lower pH. They also indicated that, as total copper increased, pH was depressed. Generally this is supported by our cold-stored sample data.

TOC was higher in the water in contact with the frozen-stored as opposed to the cold-stored sediments, indicating a release of organic matter having a potential pH buffer capacity. This buffering component possibly minimized the reactions reported by Stella and Ganzerli-Valentini [16], when an increase in cupric ion decreases pH. The higher TOC in the water column can also provide a greater potential for the copper to occur in a complexed form, creating a less toxic condition. The cold-stored peat-treated samples showed a decrease in TOC in the water, perhaps due to oxidation or decomposition. This chemical or biological decomposition appeared to be arrested by freezing. We have no explanation for the apparent decrease in TOC in the water with increasing copper concentrations.

Cupric ion activities measured at the conclusion of each test generally showed a direct relationship with total and soluble copper concentrations. Also, at any one level of bulk copper, with or without peat, the bioassay water in the cold-preserved samples had a higher ionic copper value than did their frozen counterparts. It would be difficult, however, to assign the cause of daphnid mortality to ionic copper, since our emphasis was not on speciation measurements. Since, in almost all cases, total copper was equal to soluble copper, one can infer that mortality may have been due to some soluble copper component.

We have demonstrated that the toxicity of the

cold-stored sediments was greater than that of the frozen-stored sediments. We tested only one base sediment, spiked with one form of organic matter, with only one pollutant. The results obtained may therefore be specific to those conditions. Additional testing is needed to examine the generality of these results. The levels of spiked copper and organic content used in these experiments bracket the concentrations we have recently found in sediments in the field [1,17]. Spiking procedures were chosen, however, in order to have greater control over the levels of these components.

Changes in toxicity due to long-term storage were also evaluated by conducting toxicity tests on metal-contaminated environmental sediments that had been frozen for about 2 years. These sediments had originally been tested in 1982 in beakers and recirculating Prater-Anderson devices using *D. magna*, 24-h-old or younger, during the first 2 and last 2 d of a 10-d sediment–water contact period. Since the 5-d-old *Daphnia* in the frozen-sediment tests were added after a 5-d sediment–water contact period, the results are believed to be reasonably comparable to the 8- to 10-d results of the 1982 tests. In addition, recent studies by A.V. Nebeker (personal communication) have indicated that 1- and 5-d-old *D. magna* are approximately equally sensitive to copper.

Comparisons between 1982 (fresh) and 1984 (frozen) copper-contaminated sediments from the Keweenaw Waterway of upper Michigan [17] (personal communication, M.A. Cairns) were ambiguous. In some cases, toxicity had significantly increased, and in others it had significantly decreased. However, the toxicity of sediments from a Wisconsin lake system receiving effluents from both a municipal sewer system and a metals plating plant [1] (personal communication, M.A. Cairns) was completely eliminated. These results reinforce our conclusion that freezing is undependable in preserving sediment integrity for bioassay tests.

We have demonstrated that various bioassay responses can be expected, depending on how the sediment is stored prior to testing. Both storage time and storage temperature need to be regulated.

CONCLUSIONS

Release of copper from sediment into water was attenuated in the frozen sediments. Almost all of the released copper was soluble, although significant differences in turbidity were observed. Copper release was attenuated by the presence of peat in the sediment at 5°C. Copper release into the water increased with storage time at 5°C up to week 8, then decreased. Changes in sediment appearance occurred following frozen storage. The test water became turbid and colored, and the sediment settled to one-half to two-thirds of its original volume after the test water was added. TOC in the water column above frozen-stored sediments was higher than that over cold-stored sediments. Toxicity in the frozen-stored sediments was low or absent; toxicity in the cold-stored sediments, however, was variable over time. Freezing is not recommended as a storage procedure. Cold-stored sediments should be used as soon as possible after collection.

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